(1)

TRANSLATION NO. 2304

DATE: Feb 1968

DDC AVAILABILITY NOTICE

This document has been approved for public release and sale; its distribution is unlimited.

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

(1)

TRANSLATION NO. 2304

DATE: Feb. 1966

20050202088

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

Konservnaya i Ovoschesushilnaya Promyshlennost (Food Canning and Dried Vegetables Industry), 17, No.11, pp.36 - 39, 1962.

Contemporary State of the Problem of Botulism Stimulant and Canned Food Production

By: M.N. MAZOKHINA

Central Scientific Research Institute of Canned Foods and Dried Vegetables Industry (TSNIIKOP)

(Translated by: Edward Lachowicz, Maryland, Medical-Legal Foundation, Inc., 700 Fleet Street, Baltimore, Md., 21202)

The causative agents of putrefaction that occur during canning of foodstuffs and are subjected to a thermal sterilization are most often more thermostabile than those of Cl. botulinum, thus the sterilization procedures calculated to destroy these agents will also guarantee a destruction of Cl. botulinum spores.

Nevertheless, it is essential to have a thorough knowledge of the factors that determine a possibility of the spores' germination, also of the development of botulism culture in canned foods and in nutrient media at laboratories.

There is a very long list of products, which having developed Cl. botulinum, served for a while as a source of botulism disease; this has been exposed in the course of numerous research analyses. Many investigators have determined that botulism spores can develop under specific conditions almost in all types of canned foods such as meats, fishes, vegetables and even in some fruits; sometimes, the development of botulism culture and its production of toxin may proceed without any apparent changes in the organoleptic

aspects of a product.

In most cases changes in the organoleptic aspects of canned foods are only slightly developed, if conditions are less suitable for the germination of botulism culture. Very significant in this respect are the results obtained by GREENBERG, R.A. et al. (1), who examined the effects of table salt on the formation of toxin and organoleptic changes in canned salted meats (see Table 1).

Table 1

Concentration of brine in %	Concentration (in meat) of NaCl in %	Quantities of examined specimens	Quantities of specimens in which the toxin was detected	Organoleptic indicants of meat
3.00	2.1	20	19	Spoiled meat, soft tissues, strong odor
3.02	2.2	20	19	
5.14	3.5	20	14	
6.25	4.3	118	94	Soft tissues
6.25	4.3	14	9	Soft tissues without putrefactive or cheesy odor
6.25	4.3	6	3	Firm tissues, apparent slight spoilage
7.09	5.1	97	13	Normal test-pieces
7.12	5.0	104	14	
8.95	6.4	103	0	
11.49	7.5	102	0	

It is obvious from Table 1 that increased concentration of table salt reduces the number of specimens whose production of toxin is combined with the appearance of visible putrefaction in meat.

Our examinations also revealed that, with the development of Cl. botulinum in some homogenised (for infants) vegetables, packed

in vacuum tin cans, gas protuberances have not always developed noticeably in storage.

There is an opinion that most of the European strains of Cl. botulinum type B belong to nonproteolytic cultures and they cause a putrefaction in products which is not always distinctly expressed.

The botulism microbe may be detected in many cans that were processed on a certain date. Such was the case with leek lettuce processed in Italy and sold in the USA. It is also possible that only some cans may contain botulism culture out of many millions of cans of the same product that were processed on various dates. The most frequent are instances,where in millions of cans, specimens are found that contain both, the culture and the toxin of botulism. Our experience proved that the presence of botulism spores together with vestigial microflora of canned foods may be caused by imperfections or by impairments in the production technology, or by accidental circumstances, the cause of which can not always be explained ultimately.

The detection of botulism microbe spores,that still remain in a product after thermal processing,presents familiar difficulties. One characteristic aspect of Cl. botulinum spores is a condition of dormancy,which can last up to 5 years. According to H.HIEMANN (2), Cl. botulinum spores of the same suspension may intergrow and visibly develop at random in dissimilar media (Table 2).

We determined a quantity of spores after identical inoculation into a liquid medium for cultivation of microorganisms and then we compared this with alike procedure, except we used the agar

medium in counting of colonies; we discovered that, with a prolonged storage under thermostatic control, a considerably greater number of viable spores could be detected in a liquid medium.

Table 2

Culture medium	Quantities of intergrown spores after 100 days in storage under thermostatic control	Time required (in days) for intergrowing of 50% of spores
Pork broth	12×10^6	3.4
Casein medium + yeast autolysate	11×10^6	20
Skimmed milk + 1% peptone	36×10^6	35

A detection and counting of spores that survived a heating process is especially more difficult, if processing involved an intensified heating. A medium suitable for counting of nonheated spores may not be suitable for counting of heated spores. According to the intensity of thermal processing, heated spores become more sensitive to the presence of regeneratore in a medium, to its active acidity and to the incubation temperature of inoculations.

At present, it is considered as generally acknowledged that, for the development of anaerobes, and that of Cl. botulinum as well, not the absence of oxygen is one essential condition, but a low redox potential: Eh. The oxidation-reduction potential is combined with the pH of a medium according to this formula:

$$rH_2 = \frac{Eh}{0.029} + 2pH,$$

where rH_2 is the degree of aerobic (or anaerobic) condition in a medium.

The most aerobic conditions expressed are of the magnitude:

rH_2 = 42.5; the most anaerobic conditions are: rH_2 = 0. The rH_2 value in a medium is determined on account of the presence therein of reversible oxidation-reduction pairs, also irreversible reducing agents and the action of free oxygen. For the intergrowth of Cl. botulinum spores a medium should have rH_2 no higher than 14.

The intergrowth of spores, division of cells and production of toxin in Cl. botulinum culture are combined with the medium's pH. A minimal pH value that enables the growth of Cl. botulinum culture changes considerably according to the strain and the medium as such. The lowest pH value that permits growing of Cl. botulinum is 4.8. In analyzing a case of botulism sickness that was caused by products which at the intake time possessed the pH lower than 4.5, INGRAM and R.H.M. ROBINSON (3) came to the conclusion that a high active acidity appeared in these products after production of toxin on account of the development of acid-forming micro-organisms which accompanied Cl. botulinum.

At the same time, K.I. CHERVYAKOVA (4) established a possibility of the development of Cl. botulinum in tomato sauce with active acidity above 4.5, after a prior development of micro-organisms in the sauce that alkalized the medium. The optimum pH value for the growth of Cl. botulinum culture lies between 5.5 and 7.0.

The active acidity of a medium was accepted as one of the factors on the basis of which R.G. MATHOZOVA (5) divided all canned foods currently produced in the USSR into three groups with respect to a possibility of the botulism culture development in them:

1. Canned meats, fish and natural vegetables, with the pH between 5.2 and 6.5, in which a development of Cl. botulinum occure promptly;

2. Canned foods (basically vegetable snacks), with the pH between 4.6 and 5.2, in which Cl. botulinum develope irregularly, and

3. Stewed fruits, tomato products and marinades in which Cl. botulinum does not develop.

A temperature is one of the basic factors which determine the intergrowth of spores, division of cells and production of toxin in Cl. botulinum culture. A much higher incubation temperature is required for the intergrowth of spores than for the growth and division of vegetative cells. Thus, a production of toxin at 20°C proceeds independently of the condition whether a medium was inoculated with washed-off cells or spores; vegetative cells may grow and divide at 15°C; however, washed-off spores usually seldom intergrow. The top temperature limit for the intergrowth of spores is 42.5°C.

The temperature limitations for breeding and division of cells are wider than the temperature limits for the intergrowth of spores; the former range between 10 and 48°C. The rate of the division of cells, the autolysis of culture and the synthesis of toxin, all are subject to change according to changes in the incubation temperature. P.F. BONVENTRE and L.L. KEMPE (6) stated that the maximum growth of the culture, also of the autolysis of cells and of the synthesis of toxin were observed between 28 and 40°C (see figures 1 and 2). The rate of the growth advanced with the

increased temperature, although the amount of produced toxin re-

a = optical density; b = duration of storage under thermostatic control (in hours).

Figure 1 - Dependency curves of the growth of Cl. botulinum with relation to the incubation temperature: 1 - 37°C; 2 - 35°C; 3 - 28°C; 4 - 24°C; 5 - 48°C; 6 - 10 to 18°C.

a = minimal lethal dose per 1 ml, b = duration of storage under thermostatic control (in hours).

Figure 2 - Dependency curves of the production of Cl. botulinum toxin with relation to the incubation temperature: 1 - 37°C; 2 - 35°C; 3 - 24°C; 4 - 10 to 18°C; 5 - 48°C.

mained the same. The maximum temperature at which we observed the growth of the culture was 46°C, but, at the same time, no increase

in the toxicity of substratum was noted. At this temperature, the division of vegetative cells continued, but the synthesis of toxin declined. Obviously, the toxin that was brought along with a culture and it was subsequently liberated, inactivated itself gradually by way of denaturisation. We noticed a growth of culture and synthesis of toxin at temperatures between 10 and 18°C. Since the toxin remains stable at these temperatures, a prolonged incubation is the cause of increase in the toxicity of substratum.

Substances may exist in canned foods and in nutrient media at laboratories, which inhibit the growth of botulism spores, namely: dissolved tin, fatty acids and phytoncides. The effectiveness of the bacteriostatic action of tin has been linked with certain types of canned products and with the concentration of dissolved tin.

The suppression of the intergrowth of Cl. botulinum culture under the effects of tin has been observed in experiments with carrots, beets and pod beans; no such effect was observed in experiments with asparagus, cabbage, potatoes and turnips.

Many investigators determined that some fatty acids, particularly those in a state of the oxidizing putrefaction, e.g. unsaturated (0.01% oleic, linoleic and linolenic acids) may inhibit the intergrowth of spores of Cl. botulinum and division of cells.

Fatty acids strongly suppress spores and weak vegetative cells. In a compound medium may occur fatty acids or their salts in a dissolved state in such quantities, which will exert their effect on a quantity of intergrown spores. Consequently, in re-

search experiments, e.g. in investigations of the thermostability of spores, one must take into account the influences of fatty acids in a medium. If it becomes necessary to eliminate these effects, it is proposed that 0.1% of starch be added as an inhibitor to the nutrient media.

a = Nonsterilized; b = sterilized; c = legend; d = Cl. botulinum spores not detected; e = detected not intergrown spores of Cl. botulinum; f = detected intergrown spores and Cl. botulinum toxin.

Figure 3 - Graph symbolizing heating effects on the intergrowth of Cl. botulinum spores (pH 5.5; 50,000 spores per 1 gm of product) in canned beef borsch: 1 - without heating (control); 2 - heating at 110°C for 5 minutes; 3 - heating at 120°C for 2 minutes; 4 - heating at 125°C for 1 minute.

In a number of substances which render their bactericidal and bacteriostatic effects on spores of botulism culture we also find

phytoncides. R.G. MATROZOVA experimented with tomato products and discovered phytoncides' active effects on botulism culture. The action of tomato phytoncides depended on the content therein of dry substances, which tended to combine with active reaction in a medium. The action commenced to appear with the pH 5.5 and increased noticeably with the decrease of pH to 4.5.

R.G. MATROZOVA disclosed that adding tomato paste to various vegetables that are usually served as canned foods with dinner dishes prevented a development of botulism spores therein. Likewise, R.G. MATROZOVA initiated experiments with vegetable[s] infected with nonheated spores of Cl. botulinum. Subsequent investigations of a possibility of development Cl. botulinum in former canned dinner foods were carried out in the microbiological laboratory of TSNIIKOP, beginning with the nonsterilized and sterilized beef borsch*).

Borsch inoculated with Cl. botulinum spores (figure 3) was heated at various temperatures and durations in time. Figure 3 shows that heating of Cl. botulinum spores in nonsterilized and sterilized borsch actuated the growth of botulism culture and production of toxin therein.

A dual process takes place with the heating of spores: one share of spores dies away and the other one becomes activated. It is considered that the duration of heating at which a maximum quantity of Cl. botulinum spores can be detected in a medium is equal to 0.233 minutes at 104.4°C.

---

*) - A Russian soup of several ingredients colored with red beet juice.

Thus, it is obvious from this report that, if botulism spores exist in the final microflora of canned foods, their intergrowth in a ready product, or their detection by inoculation into nutrient media, depends on many factors, which must be taken into account in connection with the microbiological controls in the canned food industry.

Literature Cited

1. GREENBERG, H.A. et al. Food Technology. 1954, vol.13, No.9. 2. RIEMANN, H.J. - Appl. Bacteriol. 1957, vol.20, No.3. - 3. INGRAM and R.H.M. ROBINSON. Proc. Soc. Appl. Bacteriol. 1951, vol.14, No.1. - 4. CHERVYAKOVA, K.I. Zhur. Mikrob., Epidemiol. i Immunol. 1941, No.10. - 5. MATROZOVA, R.G. Botulism microbe in canning industry. Moscow, Food Process. Publ., 1957. - 6. BONVENTRE, P.F. and KEMPE, L.L. Appl. Microbiol. 1959, vol. 7, No.6.

Printed footnote: Workers in industry, building construction and transportation! Implement the complex mechanization and automation! - boldly implant in industry our achievements in science, technology and in advanced experiments!

(Appeal of the Central Committee of the Communist Party on the 45th Anniversary of the Great October Revolution).